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Original communication

Distribution & diagnostic efficacy of cardiac markers CK-MB & LDH in pericardial fluid for postmortem diagnosis of ischemic heart disease

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ABSTRACT

The aim of the present study is to evaluate the diagnostic efficacy of biochemical markers creatine kinase-MB (CK-MB) and LDH in pericardial fluid for postmortem diagnosis of ischemic heart disease (IHD). We studied 119 medico-legal autopsies selected during a period of 2 years. Subjects were assigned into diagnostic groups upon final cause of death as follows: (1) sudden cardiac death due to IHD's ($n = 52$), (2) violent asphyxia ($n = 24$); (3) polytraumatic deaths ($n = 20$); (4) natural deaths excluding cardiac causes ($n = 23$). Pericardial fluid samples were tested for estimating enzyme levels. Histological examination was performed with hematoxylin and eosin (H&E) stain on myocardial tissue samples. We observed highest levels of CK-MB & LDH in deaths due to IHD's. Kruskal–Wallis test revels significant differences in activities of CK-MB ($P = 0.0001$) and LDH ($P = 0.0065$) amongst all diagnostic groups. Mann–Whitney test showed highly significant ($P < 0.0001$) levels of CK-MB in group 1 as compared to other diagnostic groups. However, LDH levels were non-discriminatory ($P = 0.0827$) between cases of IHD's and cases of other natural deaths. CK-MB levels were statistically non-significant between cases divided as myocardial infarction (MI) and severe coronary artery disease in group 1, hence its role for postmortem detection of MI is somewhat limiting. However, sensitivity and negative predictive values of its cut off level obtained in cases of IHD's are nearly equal to diagnostic efficacy in clinical settings. Hence, it can be useful additional diagnostic tool for autopsy diagnosis of IHD's. Whereas, LDH is not useful for postmortem diagnosis in these cases.

1. Introduction

Sudden, unexpected and clinically unexplained natural deaths are often a cause of perplexity to the forensic pathologists. Ischemic heart disease (IHD) due to coronary artery atherosclerosis is the most prevalent cause of sudden death in adults over the age of 30 years, but it is not infrequent in younger subjects.[1] Hence, coronary artery disease (CAD) is sometimes called 'The Captain of the Men of Death' and it constitutes a significant proportion of autopsies conducted in India.[2] More than 50% of these cases present as sudden deaths due to cardiac arrhythmias induced by myocardial ischemia. At autopsy coronary atherosclerosis without any demonstrable gross or microscopic changes of myocardial infarction (MI) is the only finding in such cases. Because, light microscopic changes of MI are not apparent within first 4–6 h. Gross changes, on the other hand, appear after 12–24 h. Hence a minimum survival period of 6 h is required for the changes of MI to appear in the heart.[3] Various histochemical techniques like hematoxylin basic fuchsin picric (HBFP) acid and the nitro blue tetrazolium (NBT) staining methods and electron or fluorescence microscopy have been adopted to identify early signs of MI. However, sensitivity and specificity of these methods are still controversial.[4,5]

Due to limitations of histopathological findings, it is necessary to establish diagnostic utility of different biochemical cardiac markers in biological fluids for postmortem diagnosis of MI. Measurements of serum cardiac isoenzyme creatine kinase-MB(CK-MB), LDH enzyme and its isoenzymes are commonly used for clinical diagnosis of acute myocardial infarction.[6–8] Few studies are reported on utility of these markers for postmortem diagnosis of MI in different biological fluids including pericardial fluid (PF).[9–18] However, they do not provide overall diagnostic efficacy of these cardiac markers for postmortem diagnosis of MI. Hence, the present study was conducted to evaluate the diagnostic

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efficacy of cardiac markers CK-MB and LDH in PF for autopsy diagnosis of MI and to analyze the distribution of these markers in different cases where the mechanism of death may affect the activities of these enzymes.

2. Material & methods

In this prospective study, total 119 cases (91 males and 28 females) were selected from medico legal autopsies conducted over a period of two years from November 2010 to October 2012 at department of Forensic Medicine, IGGMC, Nagpur. The mean age of the subjects was 50.77 years (S.D. 10.81, range 30–75 years). All dead bodies were refrigerated prior to autopsy to control postmortem artefacts. Subjects were assigned into one of the four diagnostic groups depending upon their final cause of death according to medical records, circumstances at scene of death, inquest papers, autopsy findings, toxicological analysis and complementary histological findings. In addition, history particularly important towards diagnosis of cardiac diseases was elicited from near relatives or friends. The groups were as follows: (1) sudden cardiac deaths (SCD) due to ischemic heart disease ($n = 52$) subdivided in deaths due to myocardial infarction ($n = 28$) and coronary artery disease ($n = 24$). These subgroups were classified upon histopathological confirmation; (2) violent asphyxial deaths ($n = 24$) included cases of hanging ($n = 16$) and drowning ($n = 8$); (3) polytraumatic deaths ($n = 20$) included vehicular accident cases involving extensive muscle damage; (4) natural deaths not due to cardiac diseases ($n = 23$) that included cases of pulmonary consolidation ($n = 10$), lung abscess ($n = 04$), non-traumatic intracerebral hemorrhage (ICH) ($n = 04$), non-traumatic subarachnoid hemorrhage (SAH) ($n = 02$), sepsis due to infected wounds ($n = 02$) and acute bronchial asthma (01). Cases of polytrauma were included to study the effect of muscle damage on CK-MB level in PF. Whereas; violent asphyxial deaths were included to analyze the effect of hypoxia on heart thereby affecting the enzyme levels. Natural deaths were assigned as controls. Cases with pericardial disease ($n = 21$) or hemorrhage ($n = 18$) in pericardial fluid & those showing signs of decomposition ($n = 37$) were excluded from study. All cases included in this study were within the postmortem interval of $\leq$24 h and mean postmortem intervals in Group 1, 2, 3 and 4 were 13.53 h (SD – 6.99 h, range 3–24 h), 16.41 h (SD – 7.10 h, range 4–24 h), 13.42 h (SD – 5.17 h, range 4–21 h) and 14.53 h (SD – 6.06 h, range 6–24 h), respectively.

Survival time (ST) was known in 78 cases & mean ST was 1.21 h (S.D. 1.01 h, range 0.10–5 h). All cases in group 2 were brought dead to the emergency department. Out of total 119 cases, cardio pulmonary resuscitation (CPR) was attempted in 21, 15 & 9 cases of groups 1, 3 & 4, respectively. In group 1, 43 cases had cardiac complaints out of which 21 were hospitalized and ECG findings of IHD were present in 8 cases.

Pericardial fluid samples were collected from pericardial sac by using sterile syringe after incising parietal pericardium. It was then centrifuged immediately at 5000 rpm for 15 min & supernatant was collected for analysis. Biochemical analysis of pericardial fluid was carried on an automated analyser using commercial kits under standard laboratory conditions. We used Immunoinhibition/mod. IFCC method of estimation for CK-MB and P L method for LDH, both having same principle of UV kinetic reaction.[8] On initial tests, PF showed high enzymatic activities compared to normal clinical range. Hence, dilution of fluid was carried before final analysis with normal saline (0.9%), in the ratio of 1:9 and results then obtained were multiplied by 10.

Criterion for severe coronary atherosclerosis was defined as vessel showing $\geq$75% stenosis of lumen on transverse sectioning[19] and only such cases were included in final observations. Histopathological examination (HPE) of heart was carried out in each case with haematoxylin and eosin (h&e) staining. According to protocol, labeled full thickness tissue blocks were taken from site of ischemic and necrotic (fresh and old) myocardial zones. When such findings were not evident, tissue blocks were taken from ventricular site near maximum coronary stenosis. Control samples were taken from free anterior, lateral and posterior walls of left ventricle & right ventricle & anterior and posterior wall of interventricular septum.[9,10,20,21] In the setting of coronary artery disease several focal lesions were sampled. On HPE, findings of MI were divided into acute, healing and healed infarct. Hearts showing presence of coagulative necrosis with various degrees of nuclear changes and prominent infiltration of neutrophils were diagnosed as acute MI (AMI). Whereas, cases with above changes in various stages with presence of mononuclear leucocytes and fibroblasts without neutrophils were considered as healing MI (HMI). When, HPE of hearts showed presence of collagenous scarring without cellular infiltration, cases were labeled as old healed MI (OHMI).While, presence of features like interfibrillar edema/interstitial edema and patchy eosinophila were considered as inconclusive and such cases with findings of severe coronary atherosclerosis were assigned into deaths due CAD in group 1.

3. Statistical analysis

For statistical analyses of the data, the MedCalc version 13.1.0.0 program was used. Probability level $P < 0.05$ was considered significant. Non-parametric tests i.e. Kruskal–Wallis test & Mann–Whitney test (rank–sum test) were used to compare levels of CK-MB and LDH in PF amongst four diagnostic groups & to compare pair of diagnostic groups respectively. In addition, specific contrasts for each variable grouped by diagnostic category were carried out using Mann–Whitney test. Receiver operating characteristic (ROC) curve was used for measuring area under the curve[22,23] of the variables CK-MB & LDH. By using it, diagnostic cut-off levels of both markers were obtained. The diagnostic efficacy of the test to discriminate between cases died due of IHD's and non IHD was carried out by using best cut off values of markers obtained on ROC curve.

The study protocol was approved by the Institutional Ethical Committee.

4. Results

All 52 cases included in group 1, had severe coronary atherosclerosis in one or more major epicardial arteries and triple vessel disease pattern was predominantly (48.07%) seen. Coronary thrombosis was found in 7 cases with AMI. Out of 52 cases in group 1, findings of AMI were present in 19 cases, HMI in 04 cases; AMI along with the evidence of OHMI in 05 cases and only OHMI in 14 hearts. Myocardial fibrosis & nonspecific findings like interstitial edema along with congestion were present in 02 & 08 cases, respectively. Hence, based upon findings of histopathology and dissection of heart, cases in group 1 were sub-divided into 28 cases

Table 1
Pericardial fluid Levels of CK-MB & LDH in diagnostic groups.

Enzyme	1. Ischemic heart disease		2. Violent Asphyxia	3. Polytrauma	4. Non Cardiac natural deaths
CK-MB (U/L)	Mean	4635.36.	1623.16	1088.2	858.67
	S.D.	5144.92	2166.99	1256.12	1503.65
LDH (U/L)	Mean	5870.78	5282.43	4659.35	5285.97
	S.D.	6194.43	5375.37	4729.05	3801.92

Table 2
The Mann–Whitney test showing difference in levels of enzymes in cases of definite MI & cases of CAD in group-1.

Group 1 (IHD's)	CK-MB (U/L)		LDH(U/L)
Myocardial infarction ($n = 28$)	Mean	5055	6806.3
	S.D.	5968.75	8311.18
Coronary artery disease ($n = 24$)	Mean	4144.77	4779.66
	S.D.	4050.5	1298.93
	P value	0.6729	0.7273

Table 3
Mann–Whitney test between diagnostic groups.

Variable	Comparison groups	Z statistic value	*P* value
CK-MB	1–2	4.101	0.000
	1–3	4.564	0.000
	1–4	5.538	0.000
LDH	1–2	2.173	0.0297
	1–3	3.181	0.0015
	1–4	1.735	0.0827[a]

[a] Non-significant (groups: 1 – IHD, 2 – violent asphyxia, 3 – polytrauma, 4 – natural deaths other than cardiac disease).

of definite MI excluding cases of old infarcts and remaining 24 cases as sudden deaths due to CAD. Whereas, 06 cases of violent asphyxia, 04 cases of polytrauma and 09 of natural deaths had severe CAD. Patchy OHMI was seen in two cases of hanging and myocardial fibrosis in 3 cases of natural deaths other than cardiac causes. In remaining cases congestion of the heart was predominant finding.

Table 1 shows the values (mean and S.D.) obtained for CK-MB and LDH in each diagnostic group. Highest levels of both markers were observed in death due to IHD's as compared to other diagnostic groups.

We used non-parametric Kruskal–Wallis test for comparing differences in CK-MB and LDH levels in PF amongst all diagnostic groups. We observed highly significant difference in activities of CK-MB ($P = 0.0001$) & LDH ($P = 0.0065$) amongst all four diagnostic groups. Though higher levels were obtained for both biochemical markers in cases of definite MI as compared to cases of CAD in group 1 (Table 2), however statistically results were non-significant for CK-MB ($P = 0.6729$) and LDH ($P = 0.7273$).

In the Mann–Whitney test (Table 3), we observed highly significant ($P < 0.0001$) levels of CK-MB in cases of death due IHD's when compared to deaths due to violent asphyxia, polytrauma and other natural deaths excluding cardiac causes. Whereas, LDH levels were diagnostically significant in cases of IHD's as compared to deaths due to violent asphyxia ($P = 0.0297$) and polytrauma ($P = 0.0015$).However, it's level were non-discriminatory ($P = 0.0827$) in comparison to levels found in cases of natural deaths excluding cardiac causes (group 4). Hence, results of discriminant analysis obtained on ROC curve for LDH are not included below.

5. Receiver-operating characteristic (ROC) curve analysis

For discriminant analyses, we used cause of death as grouping variable, total 119 cases in all groups were divided into deaths due to IHD's ($n = 52$) and that of due to non IHD's ($n = 67$). ROC curve (Fig. 1) was established by taking levels of CK-MB and LDH as an independent variables with paying special attention to the area (Table 4), which represents the correct diagnosis in two individuals, one with definite and probable MI and one without MI. By using ROC curve, we determined the diagnostic cut-off value of 979 U/L for CK-MB in pericardial fluid (Table 4) for post-mortem diagnosis of myocardial infarction in cases of IHD's. At this diagnostic cut off level, we have obtained sensitivity = 94.23% NPV = 94.1% & specificity = 71.64% of CK-MB for diagnosis of IHD's. No statistically significant correlations were observed between the levels of CK-MB and LDH and postmortem interval period included in the study and the use of CPR.

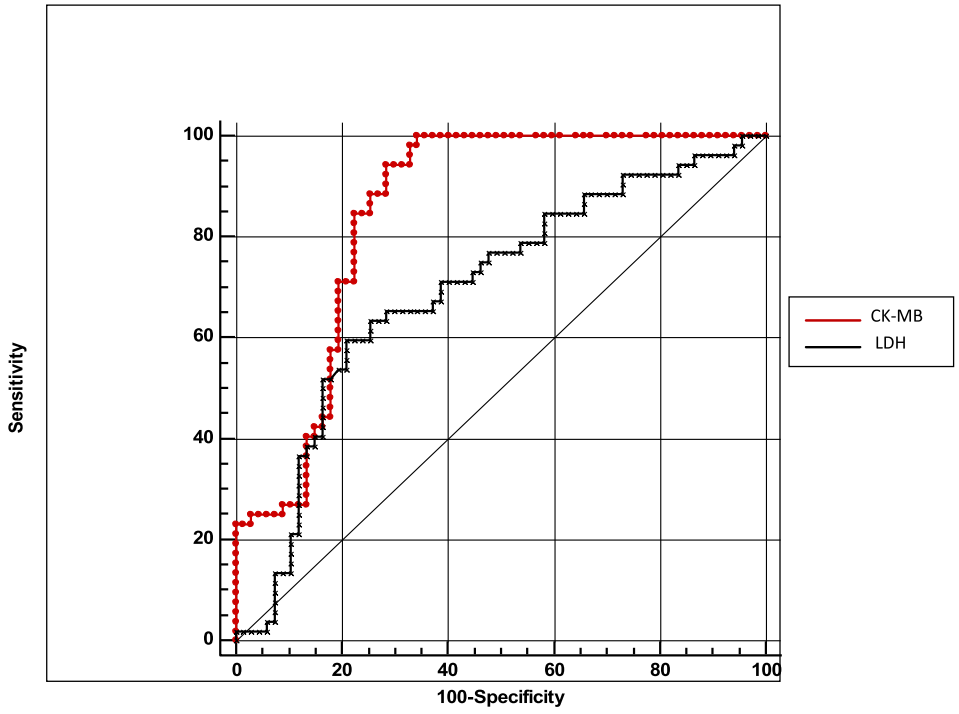

Fig. 1. ROC curve of CK-MB &LDH levels in pericardial fluid.

Table 4
Area under curve & diagnostic cut-off levels established using ROC curve analysis.

Variable	AUC[a]	*P* value	SE[b]	Lower limit	Upper limit	Cut-off level	Sensitivity	Specificity	NPV[c]
CK-MB	0.848	0.0001	0.036	0.771	0.907	979 U/L	94.23%	71.64%	94.1%
LDH	0.672	0.0007	0.050	0.580	0.755	4812.8 U/L	59.62%	77.61%	71.2%

[a] Area under curve.
[b] Standard error.
[c] Negative predictive value.

6. Discussion

In acute myocardial infarction, initial CK-MB rise occurs 4–6 h after the onset of chest pain, peaks at 24 h, and returns to baseline at 48–72 h. Whereas, level of total LDH activity in serum become elevated at 12–18 h after the onset of the symptoms, peak at 48–72 h and return to normal level in approximately 6–10 days.[6–8] In clinical practice, cardiac enzyme levels in serum are routinely used to detect myocardial ischemia. However, use of these cardiac markers in serum for postmortem diagnosis of MI has limitations due other factors affecting enzyme levels.[11,13] Hence, we chose pericardial fluid over other biological fluids, because, it is an ultrafiltrate of plasma.[24,25] Its biochemical analysis can be done by using kits standardized for serum. It lacks RBC's, therefore does not show haemolysis phenomenon that frequently interfere with biochemical determinations in serum. And as pericardial and myocardial irrigations are shared, markers of myocardial ischemia are detectable in PF before they are detectable other biological fluids.

On Kruskal–Wallis test, highly significant differences were observed for the activities of CK-MB & LDH in pericardial fluid amongst all diagnostic groups and highest values were obtained in cases of deaths due to IHD. Although slightly higher but statistically non-significant differences were noted in levels of both markers between cases of definite MI and cases with inconclusive H&E findings classified as sudden cardiac deaths due to severe coronary atherosclerosis in group 1. Raised enzyme levels in these 24 cases suggest myocardial ischemia as a triggering event in presence of severe focal coronary lesions. This signifies utility of these biochemical parameters especially CK-MB for post-mortem diagnosis of IHD's with non-specific histopathological finding. This finding from our study concur with the findings of earlier studies.[13,18]

On Mann–Whitney test, we observed statistically significant ($P < 0.0001$) levels of CK-MB in group of subjects who died of IHD's (group 1) in comparison to other groups represented by the subjects who died due to violent asphyxia (group 2), polytrauma (group 3) and natural deaths excluding cardiac causes (group 4).Similar finding were reported in earlier studies.[9,10,15,17,26] However, Barabas B[18] found non-significant difference in CK-MB levels between asphyxial deaths and death due to AMI citing intense agony prior to death as probable reason for the conflicting results. Statistically significant differences were observed in levels of LDH between group of deaths due to IHD as compared to deaths due to violent asphyxia and polytrauma excluding cases of other natural deaths included in group 4. Similar findings were noted by Carceles-Peres et al.[10] and Stuart et al.[13] On ROC curve analysis; we have observed that all the areas of the curve were significantly different from 0.50 for both CK-MB & LDH. Cut-off point levels of CK-MB showed high sensitivity (94.23%) and negative predictive value (94.1%) and only 3 cases out of 52 included as deaths due to IHD's, had levels lower than cut-off value. These were the cases of healing MI, suggestive of probable decline in enzyme levels after its initial peak during an acute phase of infarction. We observed lower specificity for CK-MB, because 19 cases of non-IHD out of 67 were incorrectly classified on discriminant analysis. This might be due to other factors affecting its levels, as out of those 19 cases, 9 corresponded to violent asphyxial deaths in which there might well have been an intense agony with consequent acute myocardial suffering involving the release of different markers into the cadaver, another 7 had died from polytraumatism in which, too, cardiac traumatism may have been involved. 3 deaths were from natural causes (2-ICH and1-SAH) that showed presence of severe coronary artery disease and death may occur due to cardiac arrhythmias in these disease conditions.[27,28] We obtained higher diagnostic sensitivity and NPV for CK-MB on ROC curve analysis in comparison with the results of Carceles-Perez et al.[10] In clinical practice, sensitivity of the serum CK-MB for diagnosis of MI is from 92 to 100 at 3 h from the onset of symptoms, whereas diagnostic specificity has been reported to be very close to 100%.[6,7]

Studies depicting, the use of ROC curve and discriminant analysis for evaluating diagnostic efficacy of LDH as cardiac markers for IHD's are not mentioned in literature. In this study, statistically non-significant difference in levels of LDH were observed between group 1 and group 4 and with diagnostic cut off value of LDH, only 31 cases out of 52 died due to IHD's were correctly classified (sensitivity = 59.62% & NPV = 71.2%). Hence, results of the present study confirming low diagnostic sensitivity of LDH for postmortem diagnosis of IHD's are in accordance with the earlier studies.[11,26]

7. Conclusions

Our study concludes that, role of CK-MB in pericardial fluid as a biochemical marker for definite detection of MI in cases of sudden cardiac death, is somewhat limiting. Because its levels were nondiscriminatory between cases of definite MI and cases of deaths due to severe CAD. However, sensitivity and negative predictive values of its cut off level obtained in pericardial fluid for postmortem diagnosis of IHD's are nearly equal to diagnostic efficacy seen on analysis of serum in clinical settings. Hence, it can be useful additional diagnostic tool for autopsy diagnosis of IHD's when histopathological examination is non-specific. Though its levels are higher, still LDH is not useful for postmortem diagnosis of MI, due to its very low sensitivity.

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Conflict of interest
None declared.

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